

REMARKS

PRIORITY

Applicants respectfully request that the Examiner hold in abeyance the request for the certified copy of the application filed in People's Republic of China on August 24, 2000 until allowable subject matter has been established.

OATH/DECLARATION

Applicants will provide a new oath or declaration when allowable subject matter has been established.

DRAWINGS

Applicants provide proposed drawings that address the size of the letters and reference characters of the Figures 1A-8 as set forth in FORM PTO-948. Notice of approval and acceptance of these figures is respectively requested.

INFORMATION DISCLOSURE STATEMENT

Applicants have submitted three Information Disclosure Statements, Form 1449, in accordance with 37 CFR 1.98(b), mailed on March 8, 2001; February 22, 2002; and July 17, 2002. All references that were listed under the Bibliography heading of the application, with the exception of general methods reference books, were listed in the first Information Disclosure Statement. The Examiner mailed copies of the March 8, 2001 and February 22, 2002 Information Disclosure Statements, signed as considered by the examiner, with office actions that were mailed on February 7, 2002 and December 18, 2002; but has not indicated that the third Information Disclosure Statement, mailed on July 17, 2002, has been considered. Thus, applicants have complied with their duty of disclosure. Accordingly, the Examiner is requested to remove the issue from this prosecution.

INTERVIEW

Applicants' representative, David R. Preston, thanks the Examiner for the courteous and informative telephonic interview of April 2, 2003. During that interview, the sum and substance of the claims and comments provided in this response were generally discussed.

THE AMENDMENTS

Applicants cancel claims 44-48, 53-56, 64-68, and 82-121, and add new claims 122 to 154. These new claims add no new subject matter and are fully supported throughout the specification and the claims as filed. For the convenience of the Examiner, a marked up copy of the new claims are provided as Attachment A. Support and reasoning for the amendments are provided below.

Support for New Claims and Reasons for Amendments

These amendments are made to clarify the claims in order to expedite allowance of the present application. Applicants reserve the right to file related applications including claims cancelled or withdrawn in this or other related applications. These claims make cosmetic changes and add no new subject matter and are fully supported throughout the specification, including the drawings and the claims as originally filed.

New independent claim 122, based on cancelled claim 82, includes the following that were included in the claims as originally filed: the one or more probes comprise DNA (from previous dependent claim 87), the survey population is a survey population of RNA molecules (from previous elected dependent claim 83, which corresponds to claim 13 of the application as filed), and the probe terminates in a known or suspected SNP (from previous elected dependent claim 85, which corresponds to claim 10 of the application as filed).

In addition, independent claim 122 recites that the probe is at least partially complementary to one or more RNAs of the survey population. This is supported throughout the specification on p.33, lines 19-22:

At least one of the probe nucleic acid molecules of the present invention is preferably at least partially complementary, or at least partially substantially complementary, to one or more nucleic acid molecules that are known to be present or are suspected of being present in a survey population of nucleic acids.

These clarifications are also supported by Figures 7A and 7B, and also stated in a paragraph discussing the embodiment depicted in Figures 7A and 7B, page 29, lines 4-13:

The set of probe nucleic acid molecules terminate at a known or suspected mutation or SNP site, and the nucleotide at the known or suspected mutation or SNP site is labeled. . . . In this embodiment, the probes are at least partially complementary or at least partially substantially complementary to the attached nucleic acid molecules that are bound to the array, and are at least partially complementary or at least partially substantially complementary to at least one nucleic acid molecule of the survey population.

Independent claim 122 has also been clarified to replace the term “nucleolytic activity” with “a nuclease that digests single-stranded DNA molecules” and to replace the term “nucleolytic activity sensitive nucleic acid molecules” with the term “non base-paired deoxynucleotides”. Support for the use of a nuclease that digests single-stranded nucleic acid molecules is particularly found in the specification on page 44, lines 17-22:

The probe nucleic acid molecule-survey population nucleic acid molecule mixture of the present invention can be treated with one or more nucleolytic activities. Nucleolytic activities of the present invention can be chemical cleavage agents, such as osmium tetroxide, hydrogen peroxide, hydroxylamine, and permanganate, or can be enzymes such as nucleases. Preferred nucleases include single-strand specific nucleases, such as S1 nuclease, Mung Bean Nuclease, Rnase T1, Rnase A, or Rnase H.

From the definition provided on page 8, lines 5 and 6 of the specification, a nucleic acid molecule can be “DNA, RNA, or a combination of both”. In a description of the embodiment depicted in Figures 7A and 7B, the specification states on page 29, lines 13-20, that:

The probe nucleic acid molecules are contacted with the survey nucleic acid molecules under conditions that promote hybridization between complementary nucleic acids, and then the probe-survey population of nucleic acid molecules is contacted with, for example, Mung Bean nuclease, a single-strand specific nuclease, such that single-stranded nucleic acid molecules are digested. Because the probes terminate in known or suspected mutation or SNP sites, their labeled termini may or may not be complementary to sequences in the survey population of nucleic acid molecules and may or may not be digested by a single-stranded nuclease . . .

Claim 122 indicates that the probe comprises DNA (as stated in the brief descriptions of the drawings for Figure 7A and Figure 7B (page 5)), so that, as illustrated in Figures 7A and 7B, as a result of treatment with “a single-stranded nuclease that digests single stranded DNA molecules”, “non-base paired deoxynucleotides are digested” (claim 122, step b).

Dependent claim 123, “wherein the terminal nucleotide at the site of said known or suspected mutation or SNP is labeled” is supported particularly by the passage on page 29, lines 4-13, cited above. It is also illustrated clearly in Figures 7A and 7B.

New dependent claims 124 and 125, indicating the type of label, are supported in the specification particularly on page 7, lines 8-19:

A probe nucleic acid molecule can optionally include a detectable label. Preferred labels include fluorochromes, such as Cy-3 and Cy-5, fluorescein, rhodamine, 7-amino-4-methylcoumarin, dansyl chloride, Hoescht 33258, R-phycoerythrin, Quantum Red (TM), Texas Red, green fluorescent protein (GFP) or other fluorescent labels as they are known or developed in the art. Alternatively, probe nucleic acid molecules of the present invention can be labeled with a radioisotope, such as ^{33}P , ^{35}S , ^3H , ^{32}P , ^{125}I , or ^{131}I . Other detectable labels that can be incorporated into a probe of the present invention include specific binding members that can be detected by other molecules that can generate a detectable signal, such as biotin. Enzymes that generate detectable signals in the presence of a suitable substrate, such as, but not limited to, alkaline phosphatase, luciferase, horseradish peroxidase, and urease can also be used as labels. Labels can optionally include mass-modified bases, that aid in distinguishing nucleic acid molecules by mass spectrometry.

New dependent claim 126, drawn to known or suspected SNPs, is supported by the passage particularly on page 29, lines 4-13, cited above.

New dependent claim 127, indicating the length of the probe, is supported by the specification particularly on p. 33, lines 15-18:

Probe nucleic acid molecules of the present invention can be of any length, but preferably are between 5 and 500 nucleoside subunits in length, more preferably between 10 and 250 nucleoside subunits in length, and most preferably between 20 and 100 nucleoside subunits in length.

New dependent claim 128, indicating that probes have one or more nuclease-resistant linkages is supported by the specification particularly on page 8, lines 19 and 20: "A probe can comprise nucleolytic-activity resistant linkages or detectable labels . . ."

Dependent claim 129, drawn to the use of two or more probes, is supported by the description on particularly page 29 of the specification, which states, "From one to four different probes can be used for each mutation or SNP to be detected . . ." (lines 6 and 7).

Dependent claims 130-132, drawn to the source of the RNA survey population, is supported by the specification particularly on page 38, lines 4-7:

The survey population of nucleic acid molecules can be comprised of RNA, of DNA, or of a combination of DNA and RNA. The DNA or RNA can be isolated from at least one cell, at least one tissue, at least one biological sample, at least one organism, or at least one environmental sample.

Dependent claim 133, indicating the type of nuclease used, is supported by the definition of "nucleolytic activity" provided particularly on page 12 of the specification, on lines 4-9:

A "nucleolytic activity" or "nucleolytic agent" is an activity that can cleave nucleosidic bonds to degrade nucleic acid molecules. Nucleolytic activities or agents can be enzymes, such as, for example, Dnase I, Exonuclease III, Mung Bean Nuclease, S1 Nuclease, RNase H, or Rnase A, or can be chemical compounds, such as hydrogen peroxide, osmium tetroxide, hydroxylamine, or potassium permanganate, or can be chemical conditions, such as high or low pH.

Dependent claims 134-136, drawn to compositions and formats for solid supports, are described particularly in the specification on page 42, line 17 through page 43, line 16. In particular:

A solid support of the present invention is a solid material having a surface for attachment of molecules, compounds, cells, or other entities. A solid support can be a membrane, such as, for example, a nylon or nitrocellulose membrane, or can be a plate or dish and can be comprised of glass, ceramics, metals, or plastics, such as, for example, a 96-well plate made of, for example, polystyrene, polypropylene, polycarbonate, or polyallomer. A solid support can also be a particle or bead that can comprise glass, can comprise one or more plastics or polymers, such as, for example, polystyrene, polyacrylamide, sepharose, agarose, cellulose or dextran, and/or can comprise metals, particularly paramagnetic metals, such as iron.

One preferred solid support of the present invention is a chip or array that comprises a flat surface, and that may comprise glass, silicon, nylon, polymers, plastics, ceramics, or metals.

Dependent claims 137-139, referring to the attached nucleic acid molecules, are supported throughout the specification. Claim 137, specifying the length of the attached nucleic acid molecules finds support on page 39, lines 22-25:

Attached nucleic acid molecules of the present invention can be of any length, but preferably are between 5 and 500 nucleoside subunits in length, more preferably between 10 and 250 nucleoside subunits in length, and most preferably between 20 and 100 nucleoside subunits in length.

Claim 138, “wherein said one or more attached nucleic acids comprise DNA or peptide nucleic acids” is supported particularly on page 38, lines 21-25:

An attached nucleic acid molecule can be RNA, DNA, or partially comprised of RNA and partially comprised of DNA. It is also within the scope of the present invention to have attached nucleic acid molecules comprising nucleic acids in which the backbone sugar is other than ribose or deoxyribose; for example, certain hexoses may be substituted. Attached nucleic acids can also be peptide nucleic acids.

Claim 139, referring to “two or more attached nucleic acid molecules” is also supported throughout the specification, including the figures, such as Figures 7A and 7B. For example, in describing various embodiments the specification refers to “a set of attached nucleic acids” (such as on page 25, line 23 and on page 27, lines 8 and 9). In describing the embodiment depicted in Figures 7A and 7B, the specification clearly indicates that more than one attached nucleic acid molecule is contemplated in stating:

“The *set* of probe nucleic acid molecules terminate at known or suspected mutation or SNP site . . . In this embodiment, the *probes* are at least partially complementary or at least partially substantially complementary to the *attached nucleic acid molecules* that are bound to the array . . .” [emphasis added]

New independent claim 140, based on cancelled claim 108, includes the following that were provided in the claims as originally filed: the one or more probes comprise DNA (from previous dependent claim 111), and the survey population is a survey population of RNA molecules (from previous elected dependent claim 109, which corresponds to claim 13 of the filed application).

Independent claim 140 has also been clarified (with respect to cancelled claim 108) to replace the term “nucleolytic activity” with “a nuclease that digests single-stranded DNA molecules” and to replace the term “nucleolytic activity-protected nucleic acid molecules” with the term “nuclease resistant nucleic acid molecules comprising one or more nuclease-protected probes hybridized to one or more survey population RNA molecules”. Support for the use of a nuclease that digests single-stranded nucleic acid molecules is found in the specification particularly on page 44, lines 17-22:

The probe nucleic acid molecule-survey population nucleic acid molecule mixture of the present invention can be treated with one or more nucleolytic activities. Nucleolytic activities of the present invention can be chemical cleavage agents, such as osmium tetroxide, hydrogen peroxide, hydroxylamine, and permanganate, or can be enzymes such as nucleases. Preferred nucleases include single-strand specific nucleases, such as S1 nuclease, Mung Bean Nuclease, Rnase T1, Rnase A, or Rnase H.

From the definition provided on page 8, lines 5 and 6 of the specification, a nucleic acid molecule can be “DNA, RNA, or a combination of both”. The description beginning on page 18, line 25 and extending to page 19, line 2, of the specification provides:

The set of probe nucleic acid molecules is contacted with the survey nucleic acid molecules under conditions that promote hybridization between complementary nucleic acids, and then the probe-survey population is contacted with a single-strand specific nuclease, such as Mung Bean nuclease, such that single-stranded nucleic acid molecules are digested.

The claim indicates that the probe comprises DNA (as stated in the brief descriptions of the drawings for Figure 1A (page 5) and shown in Figures 6A and 6B), so that, as illustrated in Figures 1A, 6A, and 6B, treatment with “a single-stranded nuclease that digests single stranded DNA molecules”, occurs such that “non-base paired deoxynucleotides are digested” (claim 140, step b).

The term “nuclease resistant nucleic acid molecules comprising one or more nuclease-protected probes hybridized to one or more survey population RNA molecules” used in step b) of claim 140 has been introduced to clarify the claim, and is supported throughout the specification, and by the Figures, particularly Figures 1A, 6A, and 6B. In the specification, for example, after the passage on pages 18 and 19 quoted above, it says: “Following nuclease treatment, the nuclease is inactivated . . . Protected probe-survey population of nucleic acid molecules are then treated . . .” (page 19, lines 3-4).

Independent claim 140 also includes “wherein said one or more attached nucleic acid molecules are at least partially complementary to said one or more probes” which is supported by the specification on page 39, line 27 to page 40, line 3:

One or more attached nucleic acid molecules of the present invention is preferably at least partially complementary, or at least partially substantially complementary, or at least partially identical, or at least partially substantially identical to at least one probe nucleic acid molecule of the present invention.

And also on page 18, lines 22-25, which describes the embodiment depicted in Figure 1A:

A set of attached nucleic acid molecules is also provided, in which the attached nucleic acid molecules are bound to a solid support in the form of an array, and in which the attached nucleic acid molecules are DNA oligonucleotides that are at least partially complementary to the probe nucleic acid molecules.

Independent claim 140 also provides: “wherein said one or more attached nucleic acid molecule/nuclease-protected probe complexes comprise single-stranded overhangs having a uniform number of bases”. This is supported by the specification, which states on page 49:

A preferred feature of the embodiments that include labeling of hybridized complexes on a solid support and that are directed toward expression profiling is that each hybridization event with a particular species of label results in a signal of the same intensity. Preferably, all four nucleotides are detectably labeled, and the number of bases to be polymerized in the extension of the nucleolytic activity-protected molecule is uniform among all the attached nucleic acid molecule/nucleolytic activity-protected complexes of the array. That is, the attached nucleic acid molecules and probe nucleic acid molecules for all positions on the array are designed such that hybridization between nucleolytic activity-protected nucleic acid molecules and attached nucleic acid molecules leaves a uniform number of bases of the nucleic acid molecules of the hybridized complexes that are not base-paired and that can be “filled in” with labeled nucleotides in polymerase reactions.

In describing the embodiment depicted in Figure 1A, the specification further states on page 19 (lines 10-14):

Attached and probe nucleic acid molecules are designed such that hybridization between complementary attached and probe nucleic acid molecules leaves single stranded overhangs on one or both ends of the hybridized complex. The number of single-stranded bases in a hybridized complex is standardized among all the possible complexes on the array.

Independent claim 140 also now refers to labeling using at least one polymerase “and at least one labeled nucleotide”, supported by the specification , for example, on page 47, lines 15-18:

In certain preferred embodiments of the present invention (such as those illustrated in **Figs. 1A, 1B, 6A, and 6B**), attached nucleic acid molecule/nucleolytic activity-protected nucleic acid molecule complexes are labeled by using one or more polymerases and one or more labeled nucleotides.

Claim 140 concludes with “detecting label incorporated into at least one of said one or more attached nucleic acid molecule/nuclease-protected probe complexes, thereby detecting one or more RNA molecules of a survey population of RNA molecules” which is supported by the specification which reads on page 58, lines 22-25:

Detection of hybridized complexes can be accomplished through any of several methods, including, but not limited to, spectrophotometric fluorescence detection, spectrophotometric absorption measurement, scintillation counting, autoradiography, phosphorimaging, light emission measurement, mass spectrometry, and the like.

And further on page 59, lines 8-14:

In detecting or visualizing the hybridization pattern, the intensity or signal value of the label can preferably be not only detected but quantified, by which is meant that the signal from each spot of the hybridization can be measured and compared to a unit value corresponding the signal emitted by known number of end labeled target nucleic acids to obtain a count or absolute value of the copy number of each end-labeled target that is hybridized to a particular spot on the array in the hybridization pattern.

And also on page 19, lines 19-26:

After washing the array, the array is scanned. Incorporation of label at a position on the array is indicative of the presence of a transcript in the survey population. The intensity of the signal at a position on the array is proportional to the number of hybridization complexes at that position, which directly reflects the number of transcripts of the gene that the attached nucleic acid molecule at that position corresponds to that are present in the survey population.

The wording of the preamble of claim 140 has also been clarified with respect to that of cancelled claim 108, on which it is based, from “a method of identifying one or more nucleic acid molecules” to “a method of detecting an RNA molecule in a survey population of RNA molecules”.

Dependent claim 141, referring to the length of the probe, is supported by the specification, particularly on p. 33, lines 15-18:

Probe nucleic acid molecules of the present invention can be of any length, but preferably are between 5 and 500 nucleoside subunits in length, more preferably between 10 and 250 nucleoside subunits in length, and most preferably between 20 and 100 nucleoside subunits in length.

Dependent claim 142, drawn to the use of more than one probe, is supported by the description on page 18 of the specification, which states: “a set of DNA probes is employed in which the probes are complementary to RNA transcripts known to be present or suspected of being present in the population.” (lines 20-22).

Claims 143 and 144, drawn to the source of the survey population of RNA molecules, is supported by the specification, particularly on page 38, lines 5-7:

The survey population of nucleic acid molecules can be comprised of RNA, of DNA, or of a combination of DNA and RNA. The DNA or RNA can be isolated from at least one cell, at least one tissue, at least one biological sample, at least one organism, or at least one environmental sample.

Claim 145, referring to the type of nuclease, is based on cancelled claim 114. Support for the use of these nucleases is found in the specification, particularly on page 12 of the specification, on lines 4-9:

A "nucleolytic activity" or "nucleolytic agent" is an activity that can cleave nucleosidic bonds to degrade nucleic acid molecules. Nucleolytic activities or agents can be enzymes, such as, for example, Dnase I, Exonuclease III, Mung Bean Nuclease, S1 Nuclease, RNase H, or Rnase A, or can be chemical compounds, such as hydrogen peroxide, osmium tetroxide, hydroxylamine, or potassium permanganate, or can be chemical conditions, such as high or low pH.

Dependent claims 146 and 147, based on cancelled claims 115 and 116, refer to the type of the solid support. Support for these claims is found in the specification, particularly on page 42, line 17 through page 43, line 16. In particular:

A solid support of the present invention is a solid material having a surface for attachment of molecules, compounds, cells, or other entities. A solid support can be a membrane, such as, for example, a nylon or nitrocellulose membrane, or can be a plate or dish and can be comprised of glass, ceramics, metals, or plastics, such as, for example, a 96-well plate made of, for example, polystyrene, polypropylene, polycarbonate, or polyallomer. A solid support can also be a particle or bead that can comprise glass, can comprise one or more plastics or polymers, such as, for example, polystyrene, polyacrylamide, sepharose, agarose, cellulose or dextran, and/or can comprise metals, particularly paramagnetic metals, such as iron. One preferred solid support of the present invention is a chip or array that comprises a flat surface, and that may comprise glass, silicon, nylon, polymers, plastics, ceramics, or metals.

Dependent claim 148, referring to the length of the attached nucleic acids, finds support in the application, for example on page 39, lines 22-25:

Attached nucleic acid molecules of the present invention can be of any length, but preferably are between 5 and 500 nucleoside subunits in length, more preferably between 10 and 250 nucleoside subunits in length, and most preferably between 20 and 100 nucleoside subunits in length.

Claim 149, "wherein said one or more attached nucleic acids comprise DNA" is supported on page 38, lines 21-22: "An attached nucleic acid molecule can be RNA, DNA, or

partially comprised of RNA and partially comprised of DNA.”

Claim 150, referring to “two or more attached nucleic acid molecules” is supported throughout the specification, including the figures, such as Figures 1A and 4.

Dependent claim 151 is based on cancelled claim 118, and is supported, for example, by the specification on page 49, lines 5-7:

Examples of DNA polymerases useful in the present invention include, but are not limited to, DNA Polymerase I, Klenow fragment, T4 DNA polymerase, T7 DNA polymerase, T. aquaticus (“Taq”) DNA polymerase, and reverse transcriptases.

Dependent claim 152-154 are drawn to the type of label and the number of labeled nucleotides. Claims 152 and 153, are supported by the specification on page 49, lines 7-9: “Polymerase reactions are performed with nucleotides, at least one of which is detectably labeled. Labels can be enzymes, specific binding members, radioisotopes, or fluorochromes.”. Claim 154 is supported by the specification on page 49, lines 16-19:

Preferably, all four nucleotides are detectably labeled, and the number of bases to be polymerized in the extension of the nucleolytic activity-protected molecule is uniform among all the attached nucleic acid molecule/nucleolytic activity-protected complexes of the array.

Claim 122 does not contain the abbreviation “SNP” without first using the full phrase

The wording of the preamble of claim 122 has been clarified with respect to that of cancelled claim 82, on which it is based, from “a method of identifying one or more nucleic acid molecules” to “a method of detecting a mutation or single nucleotide polymorphism (SNP)” to correct the informality of claim 82 which used the abbreviation “SNP” without first using the full phrase.

Claims 122-154 are definite under 35 U.S.C. 112, second paragraph.

In regard to claims 82, 97, and 108, the examiner alleges that it is unclear how “a population of nucleolytic activity-protected nucleic acid molecules can be generated if said probe-survey population mixture of nucleic acid molecules is DNA or RNA while a nuclease used in the assay is a DNase or RNase.” Applicants have cancelled these claims, and new independent claims 122 and 140 refer to the survey population being RNA and the probe comprising DNA, and replace the term “nucleolytic activity” with “at least one nuclease that digests single-stranded DNA molecules” and the term “nucleolytic activity-sensitive nucleic acid molecules with “non-base-paired deoxynucleotides”. The term “nucleolytic activity-protected nucleic acid molecules” has been replaced with “nucleic acid molecules comprising one or more nuclease-protected probes”. These changes have been made merely to clarify the claim. Applicants assert that the claim is now definite, and respectfully requests that the rejection be withdrawn.

The examiner rejects claim 94 as vague and indefinite for insufficient antecedent basis for the phrase “said known or suspected SNP or mutation”. This claim has been cancelled. Applicants respectfully request that the rejection be withdrawn.

Claims 122-139 are novel under 35 U.S.C. 102(e).

The examiner has rejected claim 82, 83, and 87-93 as allegedly being anticipated by Kris, et al., (US Patent No. 6,238,869, filed June 21, 1999, “the ‘869 patent”). Applicants have cancelled these claims and submitted new claims 122-139, where independent claim 122 is based on cancelled independent claim 82. Independent claim 122 is drawn to a method for detecting an SNP or mutation using one or more probes “. . . wherein said one or more probes terminate in known or suspected mutations”. Applicant asserts that this limitation is not disclosed in Kris et al. Examiner has stated in the office action dated Dec. 18, 2002, that a probe with substituted nucleic acids (column 4 of the ‘869 patent) constitutes a probe terminating in a mutation or SNP. However, the use of the term “substituted” in Kris, et al., means “chemically substituted”, that is, comprising alternative chemical groups, that is, having modified bases, rather than substituting

one naturally-occurring base for another naturally-occurring base, as is the case for mutations and SNPs. This is evident from the use of the term “substituted nucleic acids” in the following passage of Kris et al.:

“The nucleic acid can be modified or substituted (e.g., *comprising non naturally occurring nucleotides such as e.g., inosine*, joined via various known linkages such as sufamate, sufamide, phosphorothionate, methylphosphonate, carbamate, etc.; or a semisynthetic molecule such as a DNA-streptavidin conjugate, etc.).”

(Lines 31-36 of column 7 [emphasis added])

Thus, Kris et al. do not disclose a probe nucleic acid molecules comprising a known or suspected mutation or SNP. Applicants respectfully request that the rejection be withdrawn.

Claims 122-139 are nonobvious under 35 U.S.C. 103(a).

The examiner has rejected claim 85 as being obvious over Kris, et al., (US Patent No. 6,238,869, filed June 21, 1999). Applicants have cancelled claim 85. Applicants assert that the cited reference does not disclose all the elements of claim 122. In particular, as detailed immediately above, Kris, et al., does not disclose a probe that comprises a known or suspected mutation or SNP. Applicants therefore respectfully request that the rejection be withdrawn.

Claims 140-154 are nonobvious under 35 U.S.C. 103(a).

The examiner has rejected claims 95-97, 108, 109, 111-117, and 119-121 as allegedly obvious under 35 U.S.C. 103(a) over Kris (US Patent No. 6,238,869, “the ‘869 patent”), further in view of Zhao (US Patent No. 6,448,010, priority date October 6, 1999, “the ‘010 patent”). These claims have been cancelled. Newly submitted independent claim 140, based on cancelled claim 108, recites: “wherein said one or more attached nucleic acid molecule/nuclease-protected probe complexes comprise single-stranded overhangs having a uniform number of bases”. This aspect is not taught or suggested in Kris, et al. or Zhao. Zhao discloses the use of fill-in labeling with a polymerase to determine the identity of a base. In Zhao, determining the identity of a base requires different nucleotides that comprise different labels. This is not the case for the

embodiment of the present invention set forth in claim 140. In contradistinction to the method disclosed in Zhao, claim 140 sets forth using fill-in labeling with a polymerase to quantitatively detect an RNA molecule in a survey population. The “single-stranded overhangs having a uniform number of bases” allow for quantitative detection of nucleic acids in the present invention, is thus not relevant to the method of Zhao. Thus, Kris and Zhao do not teach or suggest all the elements of claims 140-154. Therefore applicants respectfully request that the rejection be withdrawn.

Claims 151 is nonobvious under 35 U.S.C. 103(a).

The examiner has rejected claim 118 as being obvious over Kris, et al., (US Patent No. 6,238,869, filed June 21, 1999) and Zhao (US Patent No. 6,448,010, priority date October 6, 1999, “the ‘010 patent”), further in view of Nielson et al. (US Patent No. 5,773,257, published on June 30, 1998, “the ‘257 patent”). Applicants have cancelled claim 118. New claim 151 is dependent on new independent claim 140 and recites polymerases that can be used in “labeling said one or more attached nucleic acid molecule/nuclease-protected probe complexes”. As detailed in the immediately preceeding section, applicants assert that the cited references do not disclose all the elements of independent claim 140. In particular, claim 140 recites: “wherein said one or more attached nucleic acid molecule/nuclease-protected probe complexes comprise single-stranded overhangs having a uniform number of bases”. This element is not taught or suggested in Kris, et al.; Zhao; or Nielson, et al. Applicants therefore respectfully request that the rejection be withdrawn.

Additional Remarks

Claim 98 has been cancelled. Applicants therefore respectfully request that the rejection be removed.

Applicants respectfully submit that the claims are ready for examination and in condition for allowance.

Respectfully submitted,

Date:

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David R. Preston
Reg. No. 38,710

David R. Preston & Associates, A.P.C.
12625 High Bluff Drive
Suite 205
San Diego, CA 92130
Telephone: 858.724.0375
Facsimile: 858.724.0384

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In the event this paper is deemed not timely filed the applicants hereby petition for an appropriate extension of time. The fee for this extension may be charged to Deposit Account No.501321 along with any other additional fees which may be required with respect to this paper; any overpayment should be credited to the account. If any fees charged to this Deposit Account will exceed \$500, applicant respectfully requests that its counsel be notified of such amounts before the Deposit Account is charged.

ATTACHMENT A

[82] 122. A method of [identifying one or more nucleic acid molecules] detecting a mutation or single nucleotide polymorphism (SNP), comprising:

a) contacting [at least one probe nucleic acid molecule that comprises a known or suspected SNP or mutation] one or more probes that comprise DNA with a survey population of [nucleic acid] RNA molecules under conditions that promote hybridization between complementary nucleic acid molecules to generate a probe-survey population mixture of nucleic acid molecules that comprises [one or more probe nucleic acid molecules] at least one of said one or more probes hybridized to one or more survey population [nucleic acid] RNA molecules, wherein:

said one or more probes are at least partially complementary to one or more RNA molecules known to be or suspected of being present in the survey population, further wherein said one or more probes terminate in known or suspected SNPs or mutations;

b) treating said probe-survey population mixture of nucleic acid molecules [that comprises one or more probe nucleic acid molecules hybridized to one or more survey population nucleic acid molecules] with [a nucleolytic activity] at least one nuclease that digests single-stranded DNA molecules, such that [nucleolytic activity-sensitive nucleic acid molecules] non-base-paired deoxynucleotides are digested, to generate a population of [nucleolytic activity-protected nucleic acid molecules] nucleic acid molecules comprising one or more nuclease-protected probes;

c) contacting said population of [nucleolytic activity-protected nucleic acid molecules] nucleic acid molecules comprising one or more nuclease-protected probes with a solid support that comprises one or more attached nucleic acid molecules under conditions that promote hybridization between complementary nucleic acid molecules, wherein said one or more attached nucleic acid molecules are at least partially complementary to said one or more probes, to generate one or more attached nucleic acid molecule/ [nucleolytic activity-protected nucleic acid molecule] nuclease-protected probe complexes; and

d) [identifying one or more of said attached nucleic acid molecules or one or more of said nucleolytic activity-protected nucleic acid molecules in one or more attached nucleic acid molecules/nucleolytic activity-protected nucleic acid molecule complexes] detecting at least one of said one or more attached nucleic acid molecule/probe complexes to detect a mutation or SNP.

[83. The method of claim 82, wherein said survey population comprises RNA.]

[84. The method of claim 82, wherein said survey population comprises DNA.]

[85. The method of claim 82, wherein said known or suspected SNP or mutation is at a terminus of said probe.]

[86. The method of claim 82, wherein said known or suspected SNP or mutation is not at a terminus of said probe.]

[87. The method of claim 82, wherein said at least one probe comprises DNA.]

[88. The method of claim 82, wherein said nucleolytic activity comprises at least one nuclease.]

[89. The method of claim 88, wherein said at least one nuclease is a single-strand specific nuclease.]

[93.The method of claim 82, wherein said one or more attached nucleic acid molecules are at least partially complementary to said at least one probe nucleic acid molecule.]

[94.The method of claim 82, wherein said known or suspected SNP or mutation occurs at the unattached 3' terminus of said one or more attached nucleic acid molecules.]

[95.The method of claim 82, wherein said identifying comprises labeling said attached nucleic acid molecule/nucleolytic activity-protected nucleic acid molecule complexes with at least one detectable label.]

[96.The method of claim 95, wherein said labeling uses at least one polymerase.]

[97. A method of identifying one or more nucleic acid molecules, comprising:

- a) contacting at least one probe nucleic acid molecule with a survey population of nucleic acid molecules under conditions that promote hybridization between nucleic acid molecules to generate a probe-survey population mixture of nucleic acid molecules that comprises one or more probe nucleic acid molecules hybridized to one or more survey population nucleic acid molecules;
- b) treating said probe-survey population mixture of nucleic acid molecules that comprises one or more probe nucleic acid molecules hybridized to one or more survey population nucleic acid molecules with a nucleolytic activity, such that nucleolytic activity-sensitive nucleic acid molecules are digested, to generate a population of nucleolytic activity-protected nucleic acid molecules;
- c) contacting said population of nucleolytic activity-protected nucleic acid molecules with one or more particles comprising one or more attached nucleic acid molecules under conditions that promote hybridization between nucleic acid molecules to generate attached nucleic acid molecule/nucleolytic activity-protected nucleic acid molecule complexes; and
- d) identifying one or more of said attached nucleic acid molecules or one or more of said nucleolytic activity-protected nucleic acid molecules in one or more attached nucleic acid molecule/nucleolytic activity-protected nucleic acid molecule complexes by labeling said attached nucleic acid molecule/nucleolytic activity-protected nucleic acid molecule complexes with at least one detectable label using at least one polymerase.]

[98. The method of claim 97, wherein said one or more particles is paramagnetic.]

[99. The method of claim 97, wherein said one or more particles comprises one or more polymers.]

[100. The method of claim 99, wherein at least one of said one or more polymers is polystyrene, polycarbonate, polyvinylchloride, polypropylene, polyacrylamide, sepharose, agarose, cellulose, or dextran.]

123. The method of claim 122, wherein the terminal nucleotide at the site of said known or suspected mutation or SNP is labeled.
124. The method of claim 123, wherein said label is a radioisotope, a fluorochrome, an enzyme, or a specific binding member.
125. The method of claim 124, wherein said label is a fluorochrome.
126. The method of claim 122, wherein said known or suspected SNPs or mutations are known or suspected SNPs.
127. The method of claim 122, wherein said one or more probes are from 10 to 250 bases in length.
128. The method of claim 128, wherein said one or more probes have one or more nuclease-resistant linkages.
129. The method of claim 122, wherein said one or more probes is two or more probes.
130. The method of claim 122, wherein said survey population of RNA molecules is isolated from at least one cell, at least one tissue, at least one biological sample, at least one organism, or at least one environmental sample.
131. The method of claim 130, wherein said survey population of RNA molecules is isolated from at least one biological sample or at least one environmental sample.
132. The method of claim 131, wherein said survey population of RNA molecules is isolated from at least one biological sample.

[90] 133. The method of claim [89] 122, wherein said [single-strand specific] nuclease that digests single-stranded DNA is S1 nuclease, Mung Bean nuclease, [RNase T1, RNase A], or [RNase H] Exonuclease III.

[91] 134. The method of claim [82] 122, wherein said solid support comprises glass, silicon, nylon, one or more polymers, one or more plastics, one or more ceramics, or one or more metals.

[92] 135. The method of claim [91] 134, wherein said solid support is an array.

136. The method of claim 134, wherein said solid support is a paramagnetic particle.

137. The method of claim 122, wherein said one or more attached nucleic acid molecules are between 10 and 250 nucleotides in length.

138. The method of claim 137, wherein said one or more attached nucleic acid molecules comprise DNA or peptide nucleic acids.

139. The method of claim 135, wherein said one or more attached nucleic acid molecules are two or more attached nucleic acid molecules.

[108] 140. A method of [identifying one or more nucleic acid molecules] detecting at least one RNA molecule in a survey population of RNA molecules, comprising:

a) contacting [at least one probe nucleic acid molecule] one or more probes that comprise DNA with a survey population of [nucleic acid] RNA molecules under conditions that promote hybridization between complementary nucleic acid molecules to generate a probe-survey population mixture of nucleic acid molecules that comprises one or more [probe nucleic acid molecules] probes hybridized to one or more survey population [nucleic acid] RNA molecules;

b) treating said probe-survey population mixture of nucleic acid molecules [that comprises one or more probe nucleic acid molecules hybridized to one or more survey population nucleic acid molecules] with a [nucleolytic activity] nuclease that digests single-stranded DNA molecules, [such that nucleolytic activity-sensitive nucleic acid molecules are digested,] to generate a population of [nucleolytic activity-protected] nuclease-resistant nucleic acid molecules comprising one or more nuclease-protected probes hybridized to one or more survey population RNA molecules;

c) contacting said population of [nucleolytic activity-protected] nuclease-resistant nucleic acid molecules with a solid support comprising one or more attached nucleic acid molecules, wherein said one or more attached nucleic acid molecules are at least partially complementary to said one or more probes, under conditions that promote hybridization between complementary nucleic acid molecules, to generate one or more attached nucleic acid molecule/[nucleolytic activity-protected] nuclease-protected [nucleic acid molecule] probe complexes; [and] wherein said one or more attached nucleic acid molecule/nuclease-protected probe complexes comprise single-stranded overhangs having a uniform number of bases;

d) [identifying one or more of said attached nucleic acid molecules or one or more of said nucleolytic activity-protected nucleic acid molecules in one or more attached nucleic acid molecule/nucleolytic activity-protected nucleic acid molecule complexes by] labeling said one or more attached nucleic acid molecule/ [nucleolytic activity-protected nucleic acid molecule] nuclease-protected probe complexes [with at least one detectable label] using at least one polymerase and at least one labeled nucleotide; and

e) detecting label incorporated into at least one of said one or more attached nucleic acid molecule/ nuclease-protected probe complexes, thereby detecting one or more RNA molecules of a survey population of RNA molecules .

[109. The method of claim 108, wherein said survey population comprises RNA.]

[110. The method of claim 108, wherein said survey population comprises DNA.]

[111. The method of claim 108, wherein said at least one probe comprises DNA.]

[112. The method of claim 108, wherein said nucleolytic activity comprises at least one nuclease.]

[113. The method of claim 112, wherein said at least one nuclease is a single-strand specific nuclease.]

[117. The method of claim 108, wherein said one or more attached nucleic acid molecules are at least partially complementary to said at least one probe nucleic acid molecule.]

[120. The method of claim 119, in which said at least one detectable label comprises at least one nucleotide.]

[121. The method of claim 120, wherein said at least one detectable label comprises at least two different nucleotides.]

141. The method of claim 140, wherein said one or more probes are from 10 to 250 bases in length.

142. The method of claim 140, wherein said one or more probes is more than one probe.

143. The method of claim 140, wherein said survey population of RNA molecules is isolated from at least one cell, at least one tissue, at least one biological sample, at least one organism, or at least one environmental sample.

144. The method of claim 143, wherein said survey population of RNA molecules is isolated from at least one biological sample.

[114] 145. The method of claim [113] 140, wherein said [single-strand specific nuclease] nuclease that digests single-stranded DNA molecules is S1 nuclease, Mung Bean nuclease, [Rnase T1, RNase A], or Exonuclease III [RNase H].

[115] 146. The method of claim [108] 140, wherein said solid support comprises glass, silicon, nylon, one or more polymers, one or more plastics, one or more ceramics, or one or more metals.

[116] 147. The method of claim [115] 146, wherein said solid support is an array.

148. The method of claim 140, wherein said one or more attached nucleic acid molecules are from 10 to 250 nucleotides in length.

149. The method of claim 140, wherein said attached nucleic acid molecules comprise DNA.

150. The method of claim 147, wherein said one or more attached nucleic acid molecules is two or more attached nucleic acid molecules.

[118] 151. The method of claim [108] 140, in which said at least one polymerase is one of the group comprising T4 DNA polymerase, T. aquaticus polymerase, Klenow fragment, T7 RNA polymerase, DNA polymerase I, and SP6 RNA polymerase.

[119] 152. The method of claim [108] 140, wherein said at least one [detectable label] labeled nucleotide comprises a radioisotope, a fluorochrome, an enzyme, or a specific binding member.

153. The method of claim 152, wherein said at least one labeled nucleotide comprises a fluorochrome.

154. The method of claim 152, wherein said at least one labeled nucleotide is four labeled nucleotides.